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Note: Appendices B-D are being revised by CDC. Updated versions will be forwarded when they become available.

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4. Laboratory Diagnosis of SARS

A. Goals and Key Concepts

The overall goals of SARS laboratory diagnostics are to:

- Provide the public health community with ready access to high-quality SARS diagnostics.
- Ensure that SARS laboratory diagnostics are used safely and appropriately and that results are interpreted appropriately.

The following are key concepts about SARS laboratory diagnosis:

- Specific laboratory diagnostic assays must be used to distinguish SARS from other common respiratory pathogens.
- Most patients in the early stages of SARS illness have a low titer of virus in respiratory and other secretions and require time to mount an antibody response.
- It is preferable to collect multiple specimens from different sites and at different times during illness.
- The possibility of false-positive and false-negative results with both PCR and serologic assays should always be considered when interpreting results.

B. NYSDOH Wadsworth Center Guidance Document: Laboratory Diagnosis of SARS

1. Tests that will be performed at Wadsworth Center

Tests for the SARS Coronavirus

For patients who meet the case definition and whose case history has been reviewed by the local health department (LHD) and the NYSDOH Bureau of Communicable Disease Control (BCDC), the Wadsworth Center laboratory will test for infection with the SARS coronavirus (SARS CoV) using both a reverse-transcriptase PCR (RT-PCR assay) and an enzyme-linked immunoassay (EIA). These tests have been developed by the CDC.

Tests for other viruses

The majority of SARS-like illnesses will be caused by other respiratory pathogens. Diagnosis of these infections will often make it easier to manage community anxiety about SARS. Once the presence of the SARS coronavirus has been ruled out in a clinical sample, the Wadsworth Center laboratory will perform both rapid diagnostic testing and viral culture for respiratory pathogens including influenza A and B, respiratory syncytial virus, para-influenza virus, enterovirus and adenovirus. These results will be reported to the submitting physician and, through the Bureau of Communicable Disease Control, to the LHD.

2. Samples that will not be tested

If the patient does NOT meet the case definition, testing for the SARS coronavirus will not be performed. Testing for respiratory pathogens in these patients should be performed using the normal laboratory services. If the case history has not been reviewed by staff of the Bureau of Communicable Disease Control, the LHD will be requested to follow-up

with the medical provider to determine if the patient meets the case criteria for SARS testing. Samples that are improperly labeled will not be tested until the required information is obtained. Samples with insufficient volume cannot be tested. Samples that are leaking present a safety hazard to laboratory staff and will not be tested. Samples shipped at room temperature will not be tested because of the potential for false-negative results.

3. Description of SARS tests and how they should be interpreted

The CDC has developed and validated an enzyme immunoassay (EIA) for detection of serum antibody to SARS-CoV, and a reverse transcription-polymerase chain reaction (RT-PCR) assay, for detection of SARS-CoV RNA. Both the EIA and RT-PCR tests are sensitive and highly specific for SARS-CoV. The ability to diagnose SARS-CoV infection in the patient, however, is often limited by either the low concentration of virus in most clinical specimens (RT-PCR assays) or by the time it takes a person to mount a measurable antibody response to SARS (serologic assays). The likelihood of detecting infection is increased if multiple specimens, e.g. stool, serum, and respiratory tract specimens, are collected at several times during the course of illness.

- **What does it mean if a patient with SARS has a positive test result for SARS-CoV?**

Laboratory test results should always be considered together with clinical observations and epidemiologic data in making a final diagnosis. A positive PCR result should be confirmed by a qualified second laboratory to ensure that this result is not an artifact of laboratory contamination. A positive serologic result is less likely to result from a laboratory artifact, but should also be subjected to confirmatory testing. If the results are confirmed, then a positive RT-PCR or serologic test result indicates that the patient has been recently infected with SARS-CoV (unless they have a previous history of SARS).

- **How is a SARS-CoV test confirmed?**

Positive antibody tests will be confirmed by retesting the specimens in an independent laboratory. Positive RT-PCR results will be confirmed by repeat testing of the original specimen AND by testing of the same specimen in an independent lab using a validated assay.

- **What does it mean if a patient with SARS has a negative test result for SARS-CoV?**

The only conclusive rule out test for SARS-CoV is a negative antibody result on a serum specimen collected >28 days after onset of illness. A negative antibody result on serum specimens collected ≤ 28 days after onset of illness or a negative RT-PCR test does not rule out SARS-CoV infection. Clinical specimens do not always have sufficient virus to be detected by RT-PCR and an antibody response may not be detected in some patients until >28 days after onset of illness. With the exception of a >28 day antibody test, a negative test result should not affect patient management or infection control decisions. If SARS continues to be a diagnostic concern, additional specimens should be collected and tested.

- **What does it mean if the test results are positive for other respiratory diseases?**

A positive test result for another respiratory pathogen does not rule out SARS-CoV infection. SARS patients can be co-infected with SARS-CoV and other respiratory pathogens. Thus, detection of another respiratory pathogen does not eliminate the possibility of SARS-CoV infection. In some circumstances, however, detection of another respiratory pathogen may help rule out SARS-CoV infection, e.g. the other pathogen is detected in multiple patients in a cluster of cases and can fully explain the severity of illness.

- **Should a patient with SARS who has a negative SARS-CoV test result continue to be held using isolation precautions recommended by CDC and other public health authorities?**

As noted above, the interpretation of negative SARS-CoV test results varies depending on the type of specimen, timing of specimen collection, and the test performed. With the exception of a >28 day negative serologic test result, a negative SARS-CoV test result should not affect patient isolation or management decisions. The clinical features of the illness and the type and risk of exposure are the keys to making patient management and isolation decisions.

4. Process to initiate testing

The first point of contact for a clinician who has a high suspicion for SARS in a patient is the LHD. The LHD will contact the NYSDOH Regional Epidemiologist for the area. If the LHD cannot be reached, call the NYSDOH Bureau of Communicable Disease Control at 518-473-4436 during working hours or the Duty Officer after-hours at 1-866-881-2809.

5. Method and timing of specimen collection

The CDC believes that we will maximize our ability to detect the SARS-CoV if multiple specimens are collected at different times during the illness. The following table indicates priority specimens to be collected during the course of illness. The priority is based on the likelihood that the specimen will be positive in a SARS-CoV-infected person.

Specimen	< 1 week post symptom onset	1-3 weeks post symptom onset	>3 weeks post symptom onset
Serum (serum separator tube)	++	++	++*
Blood (EDTA/purple top tube)	++	+	-
Respiratory (deep cough sputum is preferred)	++	++	+
Stool	+	++	++

* To rule out SARS serologically, it is important to collect the serum >28 days post onset

++ Priority specimen

- Not recommended

Thus, based on the chart above, the following specimens are recommended:

- Less than 1 week post-onset: collect serum, blood, respiratory secretions and stool.
- 1-3 weeks post-onset: collect serum, respiratory secretions and stool
- More than three weeks post-onset: collect serum, respiratory secretions and stool.

Key points

- It is preferable to collect multiple specimens from different sites and at different times during illness.
- Each sample should be labeled with patient's name, ID number when available, specimen type and date collected.
- The most useful respiratory specimen may be deep cough sputum.
- Nasal aspirates and lower respiratory specimens should be collected with strict attention to airborne and contact precautions, and in an airborne infection isolation room. Before collecting specimens, review infection control precautions at: http://www.cdc.gov/ncidod/sars/infectioncontrol.htm and http://www.cdc.gov/ncidod/sars/aerosolinfectioncontrol.htm.
- If patient is deceased, autopsy specimens can be submitted.
- Further guidance on specimen collection can be obtained at http://www.cdc.gov/ncidod/sars/specimen_collection_sars2.htm.

Respiratory Tract Specimens

Respiratory specimens should be collected as soon as possible in the course of the illness for most respiratory pathogens. The likelihood of recovering most viruses diminishes markedly >72 hours after symptom onset. In contrast, for SARS-CoV, the amount of virus may increase later in the course of the illness. Seven types of respiratory specimens may be collected for viral and/or bacterial diagnostics. These are: 1) nasopharyngeal wash/aspirates; 2) nasopharyngeal swabs; 3) oropharyngeal swabs; 4) broncheoalveolar lavage; 5) tracheal aspirate 6) pleural tap or 7) sputum. Deep cough sputum is preferred for detection of SARS-CoV. Nasopharyngeal wash/aspirates are the specimen of choice for detection of most respiratory viruses other than SARS-CoV and are the preferred collection method among children aged <2 years.

Upper Respiratory Tract

Collection of nasopharyngeal wash/aspirate

Collect 1-2 ml from each nostril in sterile vials using non-bacteriostatic saline. Ship with cold packs to keep sample at 4°C.

Collection of nasopharyngeal or oropharyngeal swabs

Use only sterile dacron or rayon swabs with plastic shafts. Do NOT use calcium alginate swabs or swabs with wooden sticks.

- **Nasopharyngeal swabs** - Insert swab into nostril parallel to the palate and leave in place for a few seconds to absorb secretions.
- **Oropharyngeal swabs** - Swab both posterior pharynx and tonsillar areas, avoiding the tongue.

Place both swabs immediately into same sterile vial containing 2 ml of viral transport media. Break applicator sticks off near the tip to permit tightening of the cap. Ship with cold packs to keep sample at 4°C.

Lower Respiratory Tract

Collection of broncheoalveolar lavage, tracheal aspirate, pleural tap

Collect available fluid (at least 2ml) in sterile vials with external caps and internal O-ring seals. If there are no internal O-ring seals, then seal tightly with the available cap and secure with Parafilm®. Ship with cold packs to keep sample at 4°C.

Collection of sputum

Educate the patient about the difference between sputum and spit. Have the patient rinse the mouth with water then expectorate deep cough sputum directly into a sterile screw-cap sputum collection cup or sterile dry container. Ship with cold packs to keep sample at 4°C.

Blood Components

Collection of serum for antibody or RT-PCR testing

Acute serum specimens should be collected and submitted as soon as possible. If the patient meets the case definition, convalescent specimens should be collected > 28 days after the onset of illness.

Collect 5-10 ml of whole blood in a serum separator tube. Allow blood to clot, centrifuge briefly and collect all resulting sera in vials with external caps and internal O-ring seals. If there are no internal O-ring seals, then seal tightly with the available cap and secure with Parafilm®. Label vials as containing serum with patient name, ID number and date collected. A minimum of 800 microliters of serum is preferred for each test. Ship with cold packs to keep sample at 4°C.

Pediatric patients: a minimum of 1cc of whole blood is needed for testing. If possible, collect 1cc in both an EDTA and serum separator tube. However, if only 1cc can be obtained, please use a serum separator tube for collection, spin and remove serum prior to shipping.

Collection of EDTA blood/plasma for RT-PCR

Collect 5-10 ml of blood in an EDTA (purple-top) tube. Centrifuge briefly and collect all resulting plasma in vials with external caps and internal O-ring seals. If there are no internal O-ring seals, then seal tightly with the available cap and secure with Parafilm®. Label vials as containing plasma with patient name, ID number and date collected. Ship with cold packs to keep sample at 4°C.

In order to provide the best quality specimen it is highly preferable to spin blood and remove serum or plasma prior to shipping. If it is not possible to spin blood specimens prior to shipping, ship in original collection tube sealed with Parafilm®. Blood specimens should be stored and shipped with cold packs to keep sample at 4°C. Do NOT freeze.

Collection of stool for PCR

Begin collecting stool specimens as soon as possible in the course of the illness. Although collecting earlier specimens is ideal, SARS-CoV has been detected in stool as late as one month post symptom onset. Place 10-50ml stool specimen, in a leak-proof tightly sealed stool cup or urine container. Patients may drape plastic kitchen wrap across the back half of the toilet, under the toilet seat, to facilitate collection of stool specimens. Refrigerate tubes after specimens are placed in them and ship immediately. Ship with cold packs to keep sample at 4°C.

Collection of tissue from surgical or autopsy specimens

As well as specimens described above, the CDC recommends collection of formalin fixed or paraffin embedded tissue from all major organs (lung, heart spleen, liver, brain, kidney and adrenals) and fresh frozen tissue from the lung and upper airway. Frozen tissue should be shipped on dry ice.

6. Forms to be submitted with specimens sent to Wadsworth Center

Three forms are required:

1. Wadsworth Center Virus History Form must be completed. The form is available as Attachment A.
2. Informed Consent for the RT-PCR assay.
3. Informed Consent for the EIA assay.
   The RT-PCR and EIA assays have been released by the Food and Drug Administration (FDA) under an Investigational Device Exemption and are IRB-approved. Use of both assays requires completion of two informed consent documents, one each for the RT-PCR and EIA assays. The forms are available as Attachments B and C or at the following websites. http://www.cdc.gov/ncidod/sars/lab/rtpcr/consent.htm and http://www.cdc.gov/ncidod/sars/lab/eia/consent.htm.

Note: If specimens have been collected prior to informed consent being obtained, the Patient Information Form may be completed instead of the RT-PCR and EIA informed consent forms. This form is available as Attachment D and at www://cdc.gov/ncidod/sars/lab/rtpcr/participant.htm.

The completed forms MUST be shipped with the samples. Place the completed forms in a plastic bag on top of the outer shipping container but inside the outer cardboard box before sealing.

7. Safety precautions when collecting and handling specimens

Key points

- Laboratories performing routine hematology and clinical chemistry studies should handle potential SARS specimens similarly to specimens containing other bloodborne pathogens (e.g. hepatitis or HIV, see specific biosafety guidelines at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s7f.htm).
- Laboratories performing serology or RT-PCR testing should handle potential

SARS specimens using Standard Precautions (previously Universal Precautions). Further guidance can be obtained at http://www.cdc.gov/ncidod/sars/sarslabguide.htm

- Further safety guidance for safe handling of human remains of SARS patients can be found at http://www.cdc.gov/ncidod/sars/autopsy.htm
- A detailed description of recommended facilities, practices, and protective equipment for the various laboratory biosafety levels (BSLs) can be found in the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Manual at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm.

It is estimated that several thousand diagnostic specimens from patients with SARS have been processed in routine clinical laboratories throughout the world and to date there has been no reported cases of SARS illness among laboratory workers performing diagnostic assays; however, there has been one reported case in a research laboratory setting where the SARS-CoV was being propagated. Until more information about the transmission of the SARS agent in the laboratory setting is known, precautions should be taken in handling these specimens. Effective and timely communication between clinical and laboratory staff is essential in minimizing the risk incurred in handling specimens from patients in whom SARS is suspected. Specimens from patients who may have SARS should be labeled accordingly and the laboratory should be alerted to insure proper specimen handling. Listed below are biosafety guidelines for handling these specimens.

Guidance for handling blood specimens (blood, serum, plasma)

These specimens may be handled using Standard Precautions (previously Universal Precautions). Careful attention should be given to hand hygiene after removal of gloves and especially before touching the eyes or mucosal surfaces.

Any procedure with the potential to generate fine particulate aerosols (e.g. vortexing or sonication of specimens in an open tube) should be performed in a biological safety cabinet (BSC). The use of sealed centrifuge rotors or sample cups, if available, should be employed for centrifugation. Ideally, these rotors or cups should be loaded and unloaded in a BSC. Procedures performed outside of a BSC should be performed in a manner that minimizes the risk of exposure to an inadvertent sample release.

Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against lipid-enveloped viruses should be sufficient.

Guidance for handling other specimens (e.g., respiratory secretions, stool, urine, tissue)

The following activities should be performed in BSL-2 facilities with standard BSL-2 work practices:

1. Pathologic examination and processing of formalin-fixed or otherwise inactivated tissues.

2. Molecular analysis of extracted nucleic acid preparations.
3. Electron microscopic studies with glutaraldehyde-fixed grids.
4. Routine examination of bacterial and mycotic cultures.
5. Routine staining and microscopic analysis of fixed smears.
6. Final packaging of specimens for transport to diagnostic laboratories for additional testing. Specimens should already be in a sealed, decontaminated primary container.

The following activities involving manipulation of untreated specimens should be performed in BSL-2 facilities and in a Class II biological safety cabinet:

1. Aliquoting and/or diluting specimens.
2. Inoculation of bacterial or mycological culture media.
3. Performing diagnostic tests that don't involve propagation of viral agents in vitro or in vivo.
4. Nucleic acid extraction procedures involving untreated specimens.
5. Preparation and chemical- or heat-fixing of smears for microscopic analysis.

Laboratory workers should wear protective equipment including disposable gloves and solid front gowns with cuffed sleeves. Work surfaces should be decontaminated on completion of work with appropriate disinfectants and all disposable waste autoclaved.

Any procedure or process that cannot be conducted within a biological safety cabinet requires use of the appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors). Acceptable methods of respiratory protection include a properly fit tested NIOSH approved filter respirator (N95 or higher); or powered air-purifying respirators (PAPRs) equipped with high efficiency particulate air (HEPA) filters. Accurate fit testing is a key component of effective respirator use. Personnel who cannot wear fitted respirators because of facial hair or other fit-limitations should wear loose fitting hooded or helmeted PAPRs. Centrifugation should be carried out using sealed centrifuge cups or rotors that are unloaded in a biological safety cabinet.

The following activities require BSL-3 facilities and BSL-3 work practices:

- SARS-CoV propagation in cell culture.
- Initial characterization of viral agents recovered in cultures of SARS specimens.

When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of personal protective equipment (e.g., respirators, face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) must be used.

The following activities require Animal BSL-3 facilities and BSL-3 work practices:

- Inoculation of animals for potential recovery of the agent from SARS samples.
- Protocols involving animal inoculation for characterization of putative SARS agents.

Safety procedures for safe handling of human remains of SARS patients
Postmortem procedures require adherence to standard precautions with use of appropriate personal protective equipment and facilities with appropriate safety features.

- Protective garments should include: surgical scrub suit, surgical cap, impervious gown or apron with full sleeve coverage, eye protection (goggles or face shield), shoe covers and double surgical gloves with an interposed layer of cut-proof synthetic mesh gloves.

- Respiratory protection: N-95 or N-100 respirators; or powered air-purifying respirators (PAPR) equipped with HEPA filter. Use of a PAPR is recommended for any procedures that result in mechanical generation of aerosols.

Further guidance can be obtained at http://www.cdc.gov/ncidod/sars/autopsy/htm

8. How to ship specimens to Wadsworth Center

Key points
Packaging, shipping and transport of specimens from suspect and probable SARS cases must follow the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations at http://www.iata.org/dangerousgoods/index and US DOT 49 CFR Parts 171-180 (http://hazmat.dot.gov/rules.htm)

Please note that it is the shipper's responsibility – not the transport company's - to provide the appropriate packaging materials and documentation that complies with the IATA regulations. The Wadsworth Center does not provide shipping materials.

It is essential that SARS specimens be sent to the Wadsworth Center as soon as possible after collection. If shipped within two days of collection, ship with cold packs to keep sample at 4ºC. Do not use wet ice. If shipment is delayed >2 days, then the specimens should be shipped on dry ice. Note: Whole Blood should never be sent on dry ice. All original paperwork (Attachments A-D) must be complete and accompany each specimen.

- All SARS specimens must be shipped in accordance with IATA packing instructions 650 as a "Diagnostic Specimen". Appropriate UN/IATA certified shipping materials must be used. Diagnostic specimens transported under the IATA Regulations are assigned to UN Identification number 3373. Follow the step-by-step packaging guidelines given in "Packing Diagnostic Specimens for Transport: Summary Instructions" at http://www.cdc.gov/ncidod/sars/pdf/packingspecimens-sars.pdf.
- All specimens must be sent at 4°C or, for tissue specimens, frozen on dry ice with appropriate UN certified shipping materials. If shipped within two days of collection, ship with cold packs to keep sample at 4ºC. Note: Whole Blood should never be sent on dry ice.
- All specimens must be shipped "Priority Overnight" and received within 24 hours via chosen carrier. Confirm that the selected carrier guarantees next day, morning delivery for diagnostic specimens.

- Specimens should ONLY be shipped Sunday - Thursday so that appropriate laboratory personnel can be present to accept and accession specimens Monday - Friday. Samples should not be sent to arrive on a public holiday.
- The Wadsworth Center, NYSDOH, Viral Reference and Surveillance Laboratory must be contacted via telephone or email prior to shipment. Please notify one of the following that you are shipping SARS specimens and provide patient initials and sample identification number.

  Dr. Jill Taylor 518-862-4320, jxt07@health.state.ny.us
  Dr. Norma Tavakoli 518-862-4323, npt02@health.state.ny.us
  Mr. Ryan Bennett 518-869-4551, rtc03@health.state.ny.us

Samples should be shipped to:

Shipping address: Wadsworth Center, NYSDOH
Griffin Laboratory
Virus Reference and Surveillance Laboratory
5668 State Farm Road (Route 155)
Slingerlands, NY 12159

9. When to ship specimens to Wadworth Center

At this time the Wadsworth Center laboratory will perform SARS testing only during regular working hours Monday to Friday. Specimens should therefore ONLY be shipped Sunday - Thursday so that appropriate laboratory personnel can be present to accept and accession specimens. Samples should not be shipped to arrive on a public holiday.

10. Reporting of results

SARS test information will be reported through the NYSDOH Electronic Clinical Laboratory Reporting System (ECLRS) to the LHD and a paper report sent to submitters.

Receipt of specimens

- When specimens for SARS testing are received at the Wadsworth Center, the specimen will be accessioned and entered into the clinical laboratory information management system (CLIMS).
- Once accessioned, the record will be uploaded to ECLRS by 8:00 a.m. daily. A 24/7 alert will then be generated by ECLRS to notify the NYSDOH Bureau of Communicable Disease Control (BCDC) and the LHD that a specimen for SARS testing has been received.
- If the specimen submission form does not have a Health Information Network (HIN/CDESS) ID number for the patient, NYSDOH BCDC will be notified. BCDC will contact the NYSDOH Regional Office and/or LHD for follow-up.

Test results

- Results will be uploaded to ECLRS by 8:00 a.m. each day.
- Negative results will be uploaded to ECLRS and sent to the submitter at the same time.
- Positive test results will not be uploaded to ECLRS until confirmed.
  - A 24/7 alert will be generated by ECLRS

- Preliminary positive test results
    - The initial positive test result(s) in a county will be telephoned to the NYSDOH BCDC, who will notify the NYSDOH Regional Office and LHD.

If community-transmission of SARS is documented, telephone reporting of preliminary results may need to be discontinued.

11. Contacts for additional information

Dr. Jill Taylor 518-862-4320, jxt07@health.state.ny.us
Dr. Norma Tavakoli 518-862-4323, npt02@health.state.ny.us
Mr. Ryan Bennett 518-869-4551, rtc03@health.state.ny.us

C. Roles and Activities for Laboratory Diagnosis of SARS

Clinical Laboratories and Healthcare Providers

- Report all potential SARS cases to the LHD to determine if the patient meets the surveillance case definition prior to shipping specimens to Wadsworth Center.
- Collect, handle and ship SARS specimens correctly, according to guidelines and regulations.
- Complete all appropriate forms and send to Wadsworth with the sample.
- Follow biosafety guidance to ensure that SARS specimens and SARS-CoV-infected materials are handled safely.
- Interpret SARS test results in consultation with state and/or local health department officials and with consideration of data on the clinical and epidemiologic features of the illness and the type and timing of specimen collection.

Local Health Departments

- Provide the medical provider with the case HIN ID number, guidance on specimen submission and the appropriate paperwork (Attachments A-D)
- Communicate/coordinate with the medical provider the appropriate specimen collection and submission throughout the course of the illness, including the convalescent serology.
- Educate and consult with clinicians on the interpretation of SARS diagnostics.

NYSDOH

- Conduct testing for SARS and other respiratory pathogens on specimens from patients who meet the reporting criteria.
- Ensure all positive SARS PCR test results are appropriately confirmed.
- Educate and consult with clinicians on the interpretation of SARS diagnostics.